Institut für Parasitologie
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Peter Deplazes

Strategic control of gastrointestinal nematodes in grazing sheep with a long-acting moxidectin formulation

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

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2015

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Abstract EN

A field study was undertaken on three Swiss sheep farms (A, B, C) to evaluate the efficacy of a long-acting moxidectin formulation (Cydectin® 2% LA, Zoetis) against gastrointestinal nematodes (GIN). Naturally infected ewes (all three farms) and their winter-borne lambs (farms A and B) were allocated to two groups (MOX, CON), which were grazed on separate pastures. At day 0 ewes of the MOX-groups were treated with 1 mg moxidectin s.c. Faecal and blood samples were collected at 28-day intervals. *Haemonchus contortus* was the dominant GIN-species during the grazing season in the ewes. Over the entire season the mean faecal egg count of the MOX-ewes in farms A, B, C was 56, 84 and 87% lower compared with the CON-ewes ($p<0.05$). The GIN egg output of the lambs grazing with MOX-ewes was reduced in farms A and B by 56% and 61%, respectively ($p<0.05$), compared with the respective CON-groups. None of these lambs received anthelmintic treatment during the experiment. Therefore, the differences were due to an indirect effect mediated by the lower pasture contamination with GIN-larvae. Pasture contamination was reduced by 73, 81 and 74% in farms A, B, and C respectively compared to the CON-groups ($p<0.05$). In farm B, where lambs remained with their mothers during the entire grazing season, these differences were also reflected by a higher daily weight gain ($p<0.05$) and reduced pepsinogen levels in lambs of treated ewes.

Keywords: Sheep; Moxidectin; Control; Gastrointestinal nematodes

Zusammenfassung DE

Die Wirksamkeit einer lang persistierenden Formulierung von Moxidectin (Cydectin® 2% LA, Zoetis) gegen Magen-Darmstrongyliden (MDS) wurde im Rahmen einer Feldstudie in drei Schweizer Schafbetrieben (A, B, C) geprüft. Natürlich infizierte Mutterschafe mit Lämmern (Betriebe A, B) bzw. ohne Lämmer (Betrieb C) wurden in je 2 Gruppen eingeteilt (MOX, CON), die jeweils auf getrennten Flächen weideten. Am Tag 0 wurden alle Mutterschafe der MOX-Gruppen mit 1 mg Moxidectin s.c. behandelt. Blut- und Kotproben wurden nachfolgend in 28-tägigen Abständen gesammelt. *Haemonchus contortus* war während der gesamten Saison die dominierende MDS-Spezies bei den Mutterschafen. Über die Saison gemessen war die mittlere MDS-Eiausscheidung der MOX-Mutterschafe auf den Betrieben A, B und C 56, 84 und 87% tiefer als bei den CON-Schafen ($p<0.05$%). Die MDS-Eiausscheidung der Lämmer war in den MOX-Gruppen A und B um 56 bzw. 61% gegenüber den CON-Gruppen reduziert ($p<0.05$). Da keines dieser Lämmer anthelminthisch behandelt wurde, ist dieser Effekt auf die geringere Weidekontamination mit MDS-Larven zurückzuführen. Diese war in den Betrieben A, B und C gegenüber den CON-Gruppen um 73, 81 und 74% reduziert ($p<0.05$). Auf Betrieb B, bei dem die Lämmer während der gesamten Weideperiode bei den Müttern blieben, wiesen die Lämmer der MOX-Schafe eine höhere tägliche Gewichtszunahme ($p<0.05$) und einen reduzierten Pepsinogenspiegel auf.

Schlüsselwörter: Schafe; Moxidectin; Kontrolle; Magen-Darmstrongyliden

Strategic control of gastrointestinal nematodes in grazing sheep with a long-acting moxidectin formulation

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1. Introduction

In Switzerland, as elsewhere, infections with gastrointestinal nematodes (GIN) are regarded as high priority by sheep farmers. Virtually all farms are affected and the dominance of *Haemonchus contortus* prevailing in altitudes up to 1500m may result in life-threatening disease in all age groups in the absence of adequate control measures. From May to September mean temperatures between 12 and 18°C and average monthly rainfall of 99 mm provide favourable conditions for development and migration of infective larvae (O'Connor et al., 2006). Currently, helminth control is almost entirely dependent on the use of anthelmintics. Usually, ewes and their spring-born lambs graze together until late summer. Anthelmintic treatment is usually done either at fixed intervals, or when indicated by monitoring of bulk faecal samples. For sheep kept on community pastures on which regular access to the animals is often hampered there is an increasing demand by owners to perform parasite control by long acting anthelmintics. In contrast to cattle, formulations providing a season-wide protection were not available for sheep in Europe until recently. Worldwide, only few long-acting products are registered for sheep. These include a controlled-release albendazole capsule (Proftil-Captec, Merial) (Bell and Thomas, 1992; Munyua et al., 1997) or an ivermectin capsule (IVOMEC maximize, Merial) (Gogolewski et al., 1997; Rehbein et al., 1998). Product indications suggests a protection of sheep for 100 days from reinfection with gastrointestinal helminths (Bell and Thomas, 1992; Rehbein et al., 1998) and are supposed to be a highly effective method of seasonal parasite control. However, the use of these products is limited to Australia and New Zealand and many farmers are reluctant to use intraruminal capsules because of the risk of injury by poor administration techniques (Macrae et al., 2003; Harwood and Hepple, 2011). The easy to handle pour-on formulations are not suitable for sheep as wool and skin surfaces are coated with a lipophilic emulsion of sweat and sebum. This layer can act as a solvent for chemicals and therefore diffusion of a drug within the emulsion competes with the absorption into the skin and thus limits the systemic availability (Pitman and Rostas, 1981; Rehbein, 1993; Magnusson et al., 2001; Baynes, 2004; Monteiro-Riviere et al., 2008). For these reasons an injectable long-acting anthelmintic is desirable.
Injectable ivermectin has a persistent activity for up to 10 days against reinfection with *H. contortus* at a dosage of 0.2 mg/kg (Borgsteede, 1993). Likewise, moxidectin at a dosage of 0.2mg/kg protects for up to 35 days from a reinfection with *H. contortus* and *Teladorsagia circumcincta* and 21 days from *Trichostrongylus colubriformis* (Kerboeuf et al., 1995).

Recently, a new injectable long-acting formulation of moxidectin (Cydectin 2% LA, Zoetis, Switzerland) was introduced to the market for meat sheep. For Switzerland, the manufacturer is claiming a persistent activity against reinfection with *H. contortus*, *T. circumcincta* and *T. colubriformis* for 111, 97 and 44 days, respectively.
The aim of the present study was to evaluate the efficacy of a single administration of moxidectin at a dose rate of 1mg/kg during one grazing season in three sheep farms in the Swiss midland region. In two of these farms, it was investigated if treatment of the ewes may also provide indirect protection of the untreated lambs. Furthermore, lambs could serve to maintain refugia (van Wyk, 2001) and mitigate the expansion of resistant populations given that the resistance level is still low.

2. Materials and methods

2.1 Experimental design

The study was conducted on three privately owned farms in the Swiss midland region during the 2011 grazing season. Key data of the different sites are summarised in Table 1. All ewes had been naturally infected with helminths during the previous year and all farms were free from *F. hepatica*.
On day 0, the adult sheep were randomly divided into 2 similar groups, in farm A and B based on the number of their winter-born lambs. Sheep in Farm C are neither lactating nor pregnant on day 0. Moxidectin (Cydectin 2% LA, Zoetis Switzerland) was administered subcutaneously at the base of the left ear according to the manufacturer's recommendations to the adult sheep of one group on each farm (MOX-A, MOX-B, MOX-C) at a dose rate of 1.0 mg/kg BW. Ewes of the control groups (CON-A, CON-B, CON-C) and all lambs remained untreated. On the following days, the injection site was investigated by the owner for observation of side effects.
On each farm several smaller pastures were included in the trial. Each pasture was divided into two equal paddocks, on which the groups grazed separately at equal stocking rates. Sheep were rotated between the pastures according to the same schedule for both groups and did not receive any supplement. All pastures had been grazed by sheep the previous year. Because of dry weather conditions in the early season, all groups were offered a similar portion of additional grazing area in June, which previously had been used for hay.
According to the owners' management, lambs on farms A and B were excluded from the trial at 2- 3 month of age for final indoor fattening with the exception of six lambs per group at farm A which remained in the trial to assess the indirect effect of moxidectin application to the adults on their offspring. In farms A and C, rams were kept together with the ewes to maintain breeding activities in the flocks from day 0 onwards, whereas the ram in farm B was integrated at the beginning of August. The rams in farms A and B were moved several times between the groups and were therefore kept under repeated dosing with moxidectin LA. Additionally, the absence of helminth egg shedding was controlled at monthly intervals. Parasitological data of the rams in farms A and B were not included in any analysis. To simplify description of the results, adult sheep in farm C are also referred to as ewes despite the included rams.-
One adult MOX-sheep of Farm A died in September, due to unknown reason, not related to parasitic infection. One CON-lamb of farm B disappeared at the end of June and was possibly killed by foxes. At the end of September, one clinical healthy ewe of the treated group on site C had to be removed due to misbehaviour (constant bleating) which wasn't accepted by the community living close to the pastures.

2.2 Samplings and Measurements

All animals were examined and sampled on day 0 and then at 4-weekly intervals. The sheep were clinically examined including an assessment of the colour of the eye mucosa using the FAMACHA system (van Wyk and Bath, 2002) to determine the level of anaemia. The live weight of lambs at every other sampling date was recorded. Blood samples were taken from the jugular vein for determining the packed cell volume (PCV) in all animals and pepsinogen was additionally recorded in lambs according to the method of Berghen et al.(1987). Faecal samples, directly collected from the rectum, were scored for consistency (1: watery diarrhoea; 5: well-formed dry faeces) and numbers of helminth eggs were determined quantitatively using a modified McMaster-technique(Schmidt, 1971) with a sensitivity of 50 eggs per gram (epg). Eggs other than strongyles were counted separately. Blood and faecal samples were chilled during transport and stored at 4 °C prior to processing. Third-stage larvae were cultured for each group separately according to Eckert(1960) and differentiated according to the MAFF guidelines(Ministry of Agriculture, 1986). For the individual quantitative diagnosis of lungworms, the Baermann-technique(Deplazes et al., 2012) was used. Results were expressed as larvae per 10 g of faeces. Pasture contamination with infective larvae was determined according to Sievers Prekehr(1973) modified by(Hertzberg et al., 1996).
Individual animals were treated with monepantel at a dose rate of 2.5mg/kg BW (Zolvix, Novartis, Switzerland) if their faecal egg count (FEC) exceeded 4000, PCV fell below 15 or when clinical signs of helminthosis occurred.

2.3 Meteorology

Mean 24h temperature (2m above ground level) and rainfall data were obtained from the national weather service for the whole trial period. The nearest measuring points located on a similar altitude as the specific farms were chosen resulting in an aerial distance between 3 and 24km to the farms. Collected data were compared to the averaged values from 1961-1990.

2.4 Faecal egg count reduction test and statistics

Moxidectin susceptibility was tested with the faecal egg count reduction test (FECRT), based on the recommendations of the World Association for the Advancement of Veterinary Parasitology (Coles, 1992) reviewed by Coles et al.(2006). A first series of FECRT was conducted in early April in animals not participating in the study, a second series of FECRT was envisaged at the end of the experiment.
The analyses of the FEC were done according to Torgerson et al.(2005; 2014). Remaining statistics were calculated with SPSS Statistics 21 (IBM, Switzerland). Differences in PCV and weight gain were analysed using Student's t-test. The differences in pepsinogen values, FAMACHA, faecal consistency and larval herbage counts were tested using the Wilcoxon signed rank test. Correlation between PCV and FAMACHA scores were analysed using the Spearman rho-test. All measurements were regarded as significant at a level $p<0.05$.

3. Results

Mean temperatures during the complete trial period were approximately 2 degrees Celsius higher compared to the 30-year-average. Total precipitation was 54% of the 30-year-average on farms A and B (376mm versus 687mm) whereas on farm C, it was similar to the average

(1222 mm versus 1230mm). April and May were particularly dry with only 16% and 43% average precipitation respectively. April on farm C had just 29% of average precipitation.
Arithmetic mean strongyle egg counts of the ewes were similar for MOX- and CON-groups on each farm before treatment ($p > 0.05$). Initial values were considerably higher on farms A and B with 953 and 1047 epg, respectively, compared with farm C (37 epg) (Fig. 1-3). Following treatment with long acting moxidectin at day 0, mean FEC of MOX-A- and MOX-B-ewes decreased substantially and remained significantly lower compared to those of CON-A-ewes up to the beginning ofJuly (D84) and compared with CON-B for the whole trial period apart from D82 ($p<0.05$). FEC of CON-A- and CON-B-ewes decreased markedly in early June and July, respectively, and fluctuated at a low level until the end of the trial. Over the whole season, FEC of MOX-A- and -B-ewes was 56 and 84% lower than of CON-ewes ($p<0.05$). Mean epg of MOX-C-ewes were low up to August (D105) and peaked at 65 epg on D189. In CON-C-ewes the mean FEC developed from very low levels to 490 epg on D189. Differences between CON-C- and MOX-C-ewes were significant calculated over the complete trial period with a reduction by 87% in MOX-ewes ($p<0.05$). FEC of MOX-A lambs remained below those of CON-lambs up to D 168, the difference being significant on days 28, 56, 84, 112 and 168 (Fig. 1). On farm B differences reached significance on D28 and D82 ($p<0.05$) (Fig. 2). Overall, FEC of MOX-A- and -B-lambs was reduced by 56% and 61%, respectively, compared to CON-lambs ($p<0.05$).
Apart from trichostrongyles, eggs of *Trichuris* were found on all farms, of *Capillaria* on farm A, and of *Strongyloides* on farms A and C. Such eggs were not observed in MOX-A- and MOX-C-ewes before September and never in MOX-B, whereas CON-ewes shed them over the whole trial period. In lambs, shedding of *Trichuris* and *Strongyloides* eggs started simultaneously in spring. Overall, counts were too low for statistical comparison.
Results from serial coprocultures of adult sheep are summarized in Fig. 4-6. *H. contortus* was the most abundant species in all groups until September, apart from CON-B and CON-C. In CON-B *Oesophagostomum/Chabertia* and later *Cooperia* were predominant from June onwards. In CON-C *Teladorsagia* and then *Trichostrongylus* were predominant from June onwards. In MOX-A an elevation of the *H. contortus-specific* FEC was first observed at D112. *Teladorsagia* and *Trichostrongylus* reappeared first in July and beginning of August, respectively, and *Oesophagostomum/Chabertia* and *Cooperia* in late August. In lambs, the first three months were dominated by infections with *Teladorsagia* in both experimental groups in farms A and B. Afterwards, lambs of farm A showed no consistent pattern of development of larval genera, whereas *H. contortus* became predominant on farm B. An increase of *Teladorsagia*-specific FEC was seen at D 140, when a mean value of 21 epg was found; until the end of the experiment these values remained below 60 epg. While lungworm larvae were not detected in any faecal samples on farm C, protostrongyle-larvae were found in MOX- and CON-ewes of farms A and B before treatment. After treatment protostrongyle larvae were only detected in CON-A ewes on 6 out of 8 occasions and on every sampling date in CON-B. Lungworm larvae were not found in MOX-ewes, resulting in a 100% reduction compared to CON-ewes ($p<0.05$).
Pepsinogen analysis in serum of both MOX-A- and CON-A-lambs revealed low levels around 300 mU tyrosine at day 0, differences being not significant ($p > 0.05$). Until the end of August (D140), the mean pepsinogen levels of MOX-A-lambs remained below 500 mU tyrosine and subsequently increased exceeding a mean value of 2000mU. In late October and November levels remained above those of CON-A (Fig. 7). Levels of this group rose steadily after day 0 and peaked at 1112 mU tyrosine in late August. Values of MOX-A-lambs were significantly lower on D 84, D 112 and D 140 ($p<0.05$), whereas pepsinogen levels of MOX-A-lambs were higher on D 195 and D 224 compared with CON-A-lambs ($p<0.05$). At the end of the 8-week observation period of the lambs in farm B the mean pepsinogen levels in MOX-B-lambs were significantly lower ($p<0.05$) compared with those of CON-B-lambs (70 mU tyrosine versus 432 mU tyrosine).
Mean daily weight gain of MOX-A-lambs measured over the 224 day observation period was 68g versus 45g of CON-A-lambs: an increase of 51% ($p<0.05$). Based on a measuring period of 66 days the average daily weight gain of MOX-B-lambs exceeded that of CON-B-lambs by 27% (159g versus 125g) although this difference was not significant ($p>0.05$). Measurements

of FAMACHA (Fig.8-10), PCV and faecal consistency score revealed a trend for more favourable values in the MOX-groups, but only faeces of ewes from farm C had a significantly drier consistency in moxidectin treated animals compared to controls (CON 2.8 versus MOX 3.2, $p<0.05$). FAMACHA scores and PCV were negatively correlated ($r=-0.59$ in adults and $r=-0.73$ in lambs) ($p<0.05$).
The seasonal development of pasture contamination with infective trichostrongyle larvae is shown in Table 2. For the comparison of the experimental groups data were expressed as the mean larval counts per kilogram dry matter (L3/kg DM) over the nine sampling dates. The values in MOX-A were 260 versus 1114 in CON-A ($p>0.05$) and in MOX-B 161 L3/kg DM versus 865 in CON-B ($p<0.05$) and in MOX-C 216 L3/kg DM versus 835 in CON-C ($p>0.05$), indicating a reduction of pasture larval counts on the MOX-pastures by 73, 81 and 74% in farms A, B and C respectively. Overall, apart from D28 on farm A and D49 on farm C, contamination of MOX-pastures was always below that of CON-pastures (Table 2).
FECRT's conducted in farms A and B in April exhibited a mean efficacy of 95% and 98% respectively. On farm C the test could not be done due to a very low egg excretion. After housing the FECRT performed in MOX-A-ewes showed a reduction rate of 98%, whereas due to low egg counts no corresponding test was possible in farm B. In farm C the FECRT could only be performed in CON-ewes and revealed a reduction of 80%.
Three ewes and four lambs on farm A as well as one ewe on farm B, all belonging to the control groups, had to be treated with anthelmintics because their FEC exceeded or PCV levels were less than the predetermined values. No animals from the MOX-groups had to undergo therapeutic treatment during the whole trial period. Clinical signs were seen once in a ewe of CON-B showing submandibular oedema. Adverse reactions to the moxidectin injection were not observed in any sheep.

4. Discussion

The objective of this study was to investigate the efficacy of a long-acting formulation of moxidectin in meat sheep in the Swiss midland region and to assess protection within a management system where only ewes and rams, but not lambs are treated. At day 0, mean FEC of ewes on farms A and B ranged between 800-1200 epg and were therefore adequately high for the purpose of this study. After administration of moxidectin in mid-April, MOX-ewes of farms A and B exhibited the expected decline in FEC to negligible levels and values remained low for the rest of the trial. FEC of CON-A- and -B-ewes stayed high during the first weeks of the experiment before dropping markedly at the beginning of July and June, respectively. Most likely, this drop in egg excretion in the untreated ewes was due to a self-cure phenomenon (Stoll, 1929; Gordon, 1948).This phenomenon is characterized by a spontaneous expulsion of established adult nematodes and occurs when sensitized, grazing sheep are suddenly exposed to high numbers of infective larvae on pasture. Induction of self-cure may happen after heavy summer rainfall with following liberation of trapped infective larvae from faeces (Stewart, 1950; Allonby and Urquhart, 1975), a scenario which most likely was favoured by the unusual dry conditions in April and May and mean temperatures of two degrees Celsius above average. As the ewes shed almost exclusively *H. contortus* eggs in spring, the sudden reinfection will have been mostly caused by larvae of this species and such a situation induces elimination not only of *H. contortus* but also of other abomasal species as *T. circumcincta, T. axei and T. colubriformis* (Kelly, 1973). However, the self-cure phenomenon provides no explanation for the constant low FEC in CON-ewes for the rest of the trial. Despite the overall low FEC during the second part of the season MOX-A- and -B-ewes shed 56 and 84% less eggs than the respective CON-ewes.
On farm A, the elevation of the *H. contortus*-specific FEC in MOX-ewes at D 112 is in accordance with results of Papadopoulos et al.(2009). Matching results of the same study, the first evidence for an increase of *Teladorsagia*-specific FEC was seen at D 140, however, as values remained below 100 epg until the end of the experiment conclusions concerning

efficacy of MOX during this period are limited. MOX-ewes and -lambs in farms A and B showed a trend for higher PCV values (data not shown) and lower FAMACHA scores, than CON-sheep suggesting a pathophysiological benefit of the moxidectin treatment. According to low FEC in ewes on farm B in the second part of the season, the efficacy of moxidectin, although significant for the total FEC, could not be analyzed for the different genera or species during that period. Unlike the other farms, the non-lambing ewes on farm C entered the trial with a considerably lower FEC, which was probably due to an anthelmintic treatment in the previous autumn. In contrast to the control group, the mean FEC of MOX-ewes did not increase before late autumn. The overall reduction of the FEC of 87 % compared with the CON-ewes indicated a clear benefit of the MOX-treatment. On this farm faecal consistency in the CON-group was significantly reduced compared with the MOX-sheep due to the predominance of *Teladorsagia* and *Trichostrongylus*, which in contrast to *H. contortus* are known as diarrhoea-inducing species (Sargison, 2004).
Based on the limited number of available sheep the GIN-populations on farms A and B were found to be susceptible for moxidectin before the trial. After the experiment only in farm A 4 formerly moxidectin LA-treated sheep were available for the FECRT which again revealed a mean reduction of 98% and therefore indicating no loss of efficacy throughout the experiment. In contrast, a mean of 80% efficacy was detected in CON-ewes of farm C after the experiment indicating the possible presence of anthelmintic resistant nematodes. As these animals did not have contact to macrocyclic lactones during the study, such resistant nematodes must have been present before the experiment, when due to low egg counts the resistance status could not be investigated. Apparently, despite the observed moderate reduction of efficacy of the 0.2 mg/kg formulation the long-acting formulation was able to control GIN efficiently throughout the trial period.
Under regular meteorological conditions the egg output of ewes at the beginning of the season, deriving mainly from previously hypobiotic *H. contortus*, has a substantially higher impact on subsequent infections than the overwintering pasture contamination, as *H. contortus* eggs and especially infective larvae show poor capacity of surviving on pasture during winter (Gibson and Everett, 1976; Jasmer et al., 1986; Jasmer et al., 1987). Although CON-ewes showed the highest FEC of *H. contortus* between April and June, CON-lambs shed hardly any *Haemonchus*-eggs, until July on farm B and August on farm A. Most likely the lack of precipitation diminished the survival of eggs, first and second stages of larvae of *H. contortus* and suppressed migration of infective larvae onto herbage (Stromberg, 1997; O'Connor et al., 2007), which is mirrored by the low larval counts on pastures in the first half of the season. In contrast, due to their higher capacity for overwintering (O'Connor et al., 2006) infective larvae of *Teladorsagia* were most likely present on herbage in spring, explaining the similar proportions of related infections in MOX- and CON-lambs during the first two months on pasture. Increasing pepsinogen levels in serum are indicative for abomasal damage, caused by *Teladorsagia* or *H. contortus* (Lawton et al., 1996; Simpson et al., 1997). Pepsinogen levels of MOX-A-lambs were significantly lower than the levels of CON-lambs during July and August and for MOX-B-lambs in July, the final month on pasture for that group. Four weeks after day 0, pepsinogen levels of CON-A-lambs rose to values close to 1000 mU, reflecting moderate abomasal infections. The same level was not reached by the MOX-A-lambs until October indicating a prolonged protection compared with the controls. The reason for the increasing pepsinogen levels in MOX-A-lambs between end of September and November remains unclear as these lambs experienced a lower infection pressure compared with the controls. However, this rise was associated with an elevated excretion of *Haemonchus*-eggs during October, being approximately five times higher compared with the controls, which may explain the onset of the pepsinogen rise 2-3 weeks earlier. Under practical conditions this rise would have had little impact, as most lambs are slaughtered by September. However, regular faecal examinations should be performed in lambs which are kept untreated alongside MOX-treated ewes and those which are used for stock replacement should receive an anthelmintic at the end of the season. Besides GIN low egg excretion of other nematodes, namely *Trichuris, Capillaria* and *Strongyloides* was regularly detected in CON-ewes during the whole trial, whereas in MOX-ewes they appeared only in September and in l lower numbers than in CON-ewes. Moxidectin at a dose rate of

0.2 mg/kg is known to be partially effective against *Trichuris* and *Strongyloides* in sheep (Bauer and Conraths, 1994; Coles et al., 1994) and the findings of the present study indicate that a dose rate of 1mg/kg may induce suppression of egg-shedding of these species for a period of 5 months. Furthermore, the long-acting formulation of moxidectin appears to be effective against small lungworms as in contrast to the controls, larvae were not detected in faeces in any MOX-treated ewes until termination of the study. These results are supported by findings of Papadopoulos et al. (2004) who demonstrated that moxidectin at a dose rate of 0.2 mg/kg is effective against small lungworms.
Determination of pasture larval contamination at D0 revealed overwintering of infective larvae in all farms. Similar values were obtained for both groups in farms B and C, whereas results suggested higher start values for the control group in farm A. As both experimental paddocks in this farm had been used as a common pasture the year before, this result is not explainable by the grazing history and subsequent samplings revealed low contamination on pastures of both groups. As overwintering of infective larvae is of no relevance with respect to *Haemonchus contortus* in the study region (Hertzberg, unpublished observation), the egg excretion of the adult sheep will have played the dominant role for perpetuating the infection. Despite the presence of untreated lambs the density of GIN-larvae on the MOX-pastures was clearly reduced in farms A and B. MOX-lambs profited from this situation and exhibited 56 and 61% lower FEC compared to CON-lambs in farms A and B respectively. Comparable results were obtained by Sargison et al. (2012)in a study where T. circumcincta was the dominating species. The different infection level with GIN was reflected in a significant (farm A) higher daily weight gain in MOX-A-lambs which in contrast to farm B were kept on pasture until the end of the experiment. As these lambs itself had received no anthelmintic treatment the differences are entirely due to an indirect effect mediated by the lower pasture contamination. As the GIN-infections in the CON-ewes of farms A and B decreased unexpectedly, the results provide the unexpected chance to selectively assess the impact of the period of high egg excretion after day 0 on the overall development of infections in lambs during the entire season. Therefore, the better seasonal performance of the MOX-lambs can be almost entirely attributed to the significantly lower egg excretion of the MOX-ewes during a relatively short period in spring and early summer. As a consequence it seems likely, that under usual conditions with persisting higher infections in the untreated ewes the differences of the parasitological and pathophysiological parameters between the lamb groups would have been even more pronounced. The fact that several CON-ewes and -lambs had to receive anthelmintic treatment also contributed to a less prominent divergence of the performance between both groups and reflects the higher rate of costs and labour in the unprotected animals.
When introducing long-acting anthelmintic devices on the market, the consequences with respect to selection of anthelmintic resistance have to be assessed thoroughly.

Due to slowly declining serum levels anthelmintics with persistent activity are held as an increased risk factor for the development of resistance compared to short-acting drugs (Smith et al., 1999). The so-called tail-selection allows incoming larvae with a reduced susceptibility to the drug to establish and reproduce under conditions of sub-therapeutic serum concentrations while susceptible genotypes are eliminated.
When comparing the serum levels of moxidectin after administration at a dose rate of 0.2mg/kg (recommended for moxidectin 0.1%) with those of 1mg/kg (recommended dose rate for long-acting moxidectin), the terminal slope in both kinetics is more or less parallel, indicating that by using the higher dose no prolonged exposure of the developing stages to sub-therapeutic levels occurs [R. Prichard, personal communication]. Compared with repeated applications of the lower moxidectin dose the overall number of ‘tail-events’ will be reduced in the flock assuming a single application per season per ewe as the recommended limit. Even a reduction of treatments below one per capita seems realistic, as a certain number of more resilient ewes can be left untreated as they will benefit from a reduced pasture contamination. In a *Haemonchus*-dominated environment, as in the present study the FAMACHA-scoring is a suitable approach for identification of anaemic individuals (van

Wyk and Bath, 2002; Kaplan et al., 2004). This was supported by the significant negative correlation between FAMACHA scores and PCV in the present study.
A long withdrawal period for meat, which in the case of Switzerland amounts to 104 days, and the manufacturer's restriction to use moxidectin LA in lambs weighing less than 15 kg, will exclude most of the offspring which is scheduled for slaughter during the second part of the grazing season from treatment with this formulation. The observation that lambs can be indirectly protected by the reduced egg output of the ewes could mitigate this deficit.
When multiple treatments of both lambs and ewes can be replaced by a single treatment of the ewes with a long-acting product, this strategy would confine the risk for selection of resistant GIN to the ewes. By reducing the number of 'tail-events' on the flock level this strategy may therefore offer an interesting potential for expanding refugia. As a consequence of the relatively small farm sizes and limited number of animals included in the present study further research is needed to support this concept and to perform comparative studies with other established strategic control strategies.

Acknowledgements

The support of the sheep farmers participating in the study is greatly acknowledged. The authors are indebted to Zoetis, Switzerland for financial support of this study. MeteoSwiss kindly provided the meteorological data. The valuable technical assistance of Mrs. Lucy Kohler is greatly acknowledged.

References

Allonby, E.W., Urquhart, G.M., 1975. The epidemiology and pathogenic significance of haemonchosis in a merino flock in East Africa. Veterinary Parasitology 1, 129-143.

Bauer, C., Conraths, F.J., 1994. Comparative efficacy of moxidectin and mebendazole against gastrointestinal nematodes in experimentally infected lambs. Vet Rec 135, 136-138.

Baynes, R.E., 2004. In vitro dermal disposition of abamectin (avermectin B< sub> 1</sub>) in livestock. Research in veterinary science 76, 235-242.

Bell, S.L., Thomas, R.J., 1992. The efficacy of an albendazole intraruminal controlled-release device against gastrointestinal parasitism in lambs. Veterinary Parasitology 41, 233-239.

Berghen, P., Dorny, P., Vercruysse, J., 1987. Evaluation of a simplified blood pepsinogen assay. Am J Vet Res 48, 664-669.

Borgsteede, F.H., 1993. The efficacy and persistent anthelmintic effect of ivermectin in sheep. Vet Parasitol 50, 117-124.

Coles, G.C., Bauer, C., Borgsteede, F. H., Geerts, S., Klei, T. R., Taylor, M. A., Waller, P. J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology 44, 35-44.

Coles, G.C., Giordano-Fenton, D.J., Tritschler, J.P., 2nd, 1994. Efficacy of moxidectin against nematodes in naturally infected sheep. Vet Rec 135, 38-39.

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology 136, 167-185.

Deplazes, P., Eckert, J., von Samson-Himmelstjerna, G., Zahner, H., 2012. Lehrbuch der Parasitologie für die Tiermedizin, Enke.

Eckert, J., 1960. Die Diagnose des Magen-Darm-Strongylidenbefalls des Schafes durch Differenzierung der freilebenden dritten Larven. Zentralbl. Veterinaermed. 7, 612-630.

Gibson, T.E., Everett, G., 1976. The ecology of the free-living stages on Haemonchus contortus. The British veterinary journal 132, 50-59.

Gogolewski, R.P., Allerton, G.R., Rug, D., Barrick, R.A., Eagleson, J.S., 1997. Control of gastro-intestinal parasitism in sheep with ivermectin delivered via an intraruminal controlled-release capsule. New Zealand Veterinary Journal 45, 50-56.

Gordon, H.M., 1948. The epidemiology of parasitic diseases, with special reference to studies with nematode parasites of sheep. Australian Veterinary Journal 24, 17-45.

Harwood, D., Hepple, S., 2011. Drenching/bolus gun injuries in sheep. Veterinary Record 168, 308-309.

Hertzberg, H., Durgiai, B., Schnieder, T., Kohler, L., Eckert, J., 1996. Prophylaxis of bovine trichostrongylidosis and dictyocaulosis in the alpine region: comparison of an early and late administration of the oxfendazole pulse release bolus to first year grazing calves. Veterinary Parasitology 66, 181-192.

Jasmer, D.P., Wescott, R.B., Crane, J.W., 1986. Influence of cold temperatures upon development and survival of eggs of Washington isolates of Haemonchus contortus and Ostertagia circumcincta, Helminthological Society of Washington, Washington, DC, USA.

Jasmer, D.P., Wescott, R.B., Crane, J.W., 1987. Survival of third-stage larvae of Washington isolates of Haemonchus contortus and Ostertagia circumcincta exposed to cold temperatures. Proceedings of the Helminthological Society of Washington 54, 48-52.

Kaplan, R.M., Burke, J.M., Terrill, T.H., Miller, J.E., Getz, W.R., Mobini, S., Valencia, E., Williams, M.J., Williamson, L.H., Larsen, M., Vatta, A.F., 2004. Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. Veterinary Parasitology 123, 105-120.

Kelly, J.D., 1973. Immunity and epidemiology of helminthiasis in grazing animals. N Z Vet J 21, 183-194.

Kerboeuf, D., Hubert, J., Cardinaud, B., Blond-Riou, F., 1995. The persistence of the efficacy of injectable or oral moxidectin against Teladorsagia, Haemonchus and Trichostrongylus species in experimentally infected sheep. Veterinary Record 137, 399-401.

Lawton, D.E., Reynolds, G.W., Hodgkinson, S.M., Pomroy, W.E., Simpson, H.V., 1996. Infection of sheep with adult and larval Ostertagia circumcincta: effects on abomasal pH and serum gastrin and pepsinogen. Int J Parasitol 26, 1063-1074.

Macrae, A.I., Barnes, D.F., Hunter, H.A.A., Sargison, N.D., Scott, P.R., Blissitt, K.J., Booth, T.M., Pirie, R.S., 2003. Diagnosis and treatment of retropharyngeal injuries in lambs associated with the administration of intraruminal boluses. Veterinary Record 153, 489-492.

Magnusson, B.M., Walters, K.A., Roberts, M.S., 2001. Veterinary drug delivery: potential for skin penetration enhancement. Advanced Drug Delivery Reviews 50, 205-227.

Ministry of Agriculture, F.a.F., 1986. Manual of Veterinary Parasitological Techniques: Reference Book 418 HMSO, London.

Monteiro-Riviere, N.A., Baynes, R.E., Riviere, J.E., 2008. Animal skin morphology and dermal absorption. Dermal Absorption and Toxicity Assessment, 17-36.

Munyua, W.K., Githigia, S.M., Mwangi, D.M., Kimoro, C.O., Ayuya, J.M., 1997. The Effects of a Controlled-Release Albendazole Capsule (Proftril-Captec) on Parasitism in Grazing Corriedale Ewes in the Nyandarua District of Kenya. Veterinary Research Communications 21, 85-99.

O'Connor, L.J., Kahn, L.P., Walkden-Brown, S.W., 2007. The effects of amount, timing and distribution of simulated rainfall on the development of Haemonchus contortus to the infective larval stage. Vet Parasitol 146, 90-101.

O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. Vet Parasitol 142, 1-15.

Papadopoulos, E., Sotiraki, S., Himonas, C., Fthenakis, G.C., 2004. Treatment of small lungworm infestation in sheep by using moxidectin. Vet Parasitol 121, 329-336.

Papadopoulos, E., Sotiraki, S., Himonas, C., Fthenakis, G.C., 2009. Persistent efficacy of long-acting moxidectin for control of trichostrongylid infections of sheep. Small Ruminant Research 81, 171-173.

Pitman, I.H., Rostas, S.J., 1981. Topical drug delivery to cattle and sheep. Journal of Pharmaceutical Sciences 70, 1181-1193.

Rehbein, S., 1993. Untersuchungen zur anthelminthischen Wirksamkeit von verschiedenen Ivermectin-Formulierungen gegenüber Magen-Darm-Nematoden bei experimentell infizierten Schafen und Ziegen. Universität Leipzig.

Rehbein, S., Batty, A.F., Barth, D., Visser, M., Timms, B.J., Barrick, R.A., Eagleson, J.S., 1998. Efficacy of an ivermectin controlled-release capsule against nematode and arthropod endoparasites in sheep. Veterinary Record 142, 331-334.

Sargison, N., 2004. Differential diagnosis of diarrhoea in lambs. In Practice 26, 20-27.

Sargison, N.D., Bartram, D.J., Wilson, D.J., 2012. Use of a long acting injectable formulation of moxidectin to control the periparturient rise in faecal Teladorsagia circumcincta egg output of ewes. Veterinary Parasitology 189, 274-283.

Schmidt, U., 1971. Parasitologische Kotuntersuchung durch ein neues Verdünnungsverfahren. Tierärztliche Umschau 26, 229-230.

Sievers Prekehr, G.H.C., 1973. Methode zur Gewinnung von 3. Strongylidenlarven aus dem Weidegras. Vet.Med. thesis.School of veterinary Medicine, Hannover.

Simpson, H.V., Lawton, D.E., Simcock, D.C., Reynolds, G.W., Pomroy, W.E., 1997. Effects of adult and larval Haemonchus contortus on abomasal secretion. Int J Parasitol 27, 825-831.

Smith, G., Grenfell, B.T., Isham, V., Cornell, S., 1999. Anthelmintic resistance revisited: under-dosing, chemoprophylactic strategies, and mating probabilities. Int J Parasitol 29, 77-91; discussion 93-74.

Stewart, D.F., 1950. Studies on resistance of sheep to infestation with Haemonchus contortus and Trichostrongylus spp. and on the immunological reactions of sheep exposed to infestation. IV. The antibody response to natural infestation in grazing sheep and the 'self-cure' phenomenon. Australian Journal of Agricultural Research 1, 427-439.

Stoll, N.R., 1929. Studies with the strongyloid nematode haemonchus contortus. American Journal of Epidemiology 10, 384-418.

Stromberg, B.E., 1997. Environmental factors influencing transmission. Vet Parasitol 72, 247-256; discussion 257-264.

Torgerson, P.R., Paul, M., Furrer, R., 2014. Evaluating faecal egg count reduction using a specifically designed package “eggCounts” in R and a user friendly web interface. International Journal for Parasitology 44, 299-303.

Torgerson, P.R., Schnyder, M., Hertzberg, H., 2005. Detection of anthelmintic resistance: a comparison of mathematical techniques. Veterinary Parasitology 128, 291-298.

van Wyk, J.A., Bath, G.F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet Res 33, 509-529.

Tables and Figures

Table 1:

Basic data on location, animals and treatments in the three experimental flocks

	Farm A	**Farm B**	**Farm C**
Geographic latitude and longitude	47°53' N/8°22' E	47°46' N/8°25' E	47°39' N/8°85' E
Height above sea level (m)	370	470	593
Sheep breed	mixed meat breeds	Shropshire	Swiss White Alpine
Trial period	12 April- 22 Nov.	14 April-24 Nov.	26 April-1 Nov.
Date of administration of moxidectin LA	12 April	14 April	26 April
Number of ewes	10[a]/10[b]	7[a]/8[b]	11[a]/11[b]
Number of lambs	10[a]/8[b] (6[a]/6[b])[c]	9[a]/6[b, d]	-
Number of paddocks per group	8	4	4

[a] MOX group

[b] CON group

[c] number of lambs remaining in the trial until the end, (in brackets, only farm A)

[d] lambs remained in the trial for only 3 months

Table 2:

Total number of infective third-stage trichostrongyle larvae per kg dried herbage on pastures

		Farm A				Farm B				Farm C	
		MOX	CON			MOX	CON			MOX	CON
D0	12 April	0	1600	D0	14 April	124	119	D0	26 April	1134	1334
D28	10 May	41	28	D26	10 May	189	582	D21	17 May	51	135
D56	07 June	0	137	D54	07 June	49	452	D49	14 June	267	262
D84	05 July	22	78	D82	05 July	37	67	D77	12 July	0	199
D112	02 Aug.	36	692	D111	03 Aug.	82	540	D105	09 Aug.	0	68
D140	30 Aug.	59	146	D138	30 Aug.	58	227	D133	06 Sept.	15	38
D168	27 Sept.	485	694	D166	27 Sept.	310	1829	D161	04 Oct.	189	4398
D195	24 Oct.	443	713	D194	25 Oct.	203	942	D189	01 Nov.	70	248
D224	22 Nov.	1252	5938	D224	24 Nov.	392	3029				

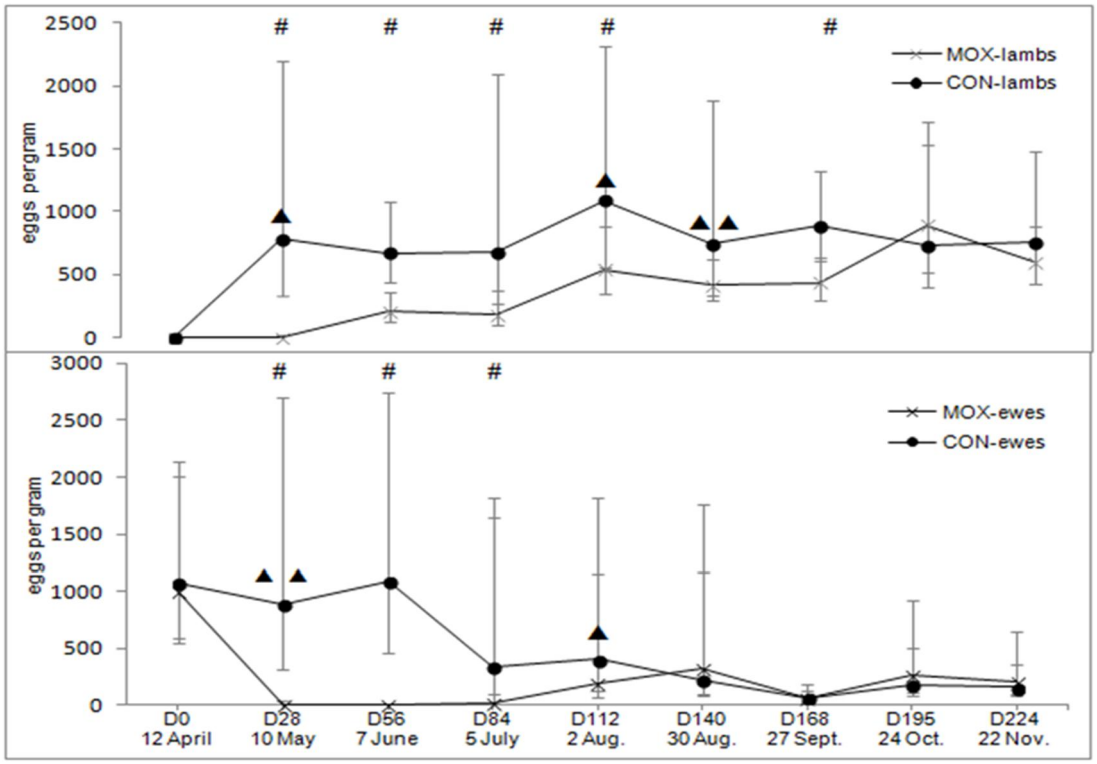
Figure 1-3: Mean faecal trichostrongyle egg counts of ewes and lambs

▲: each symbol represents a salvage treatment
#: differences between MOX and CON significant ($p<0.05$)

Fig. 1:

a)

b)

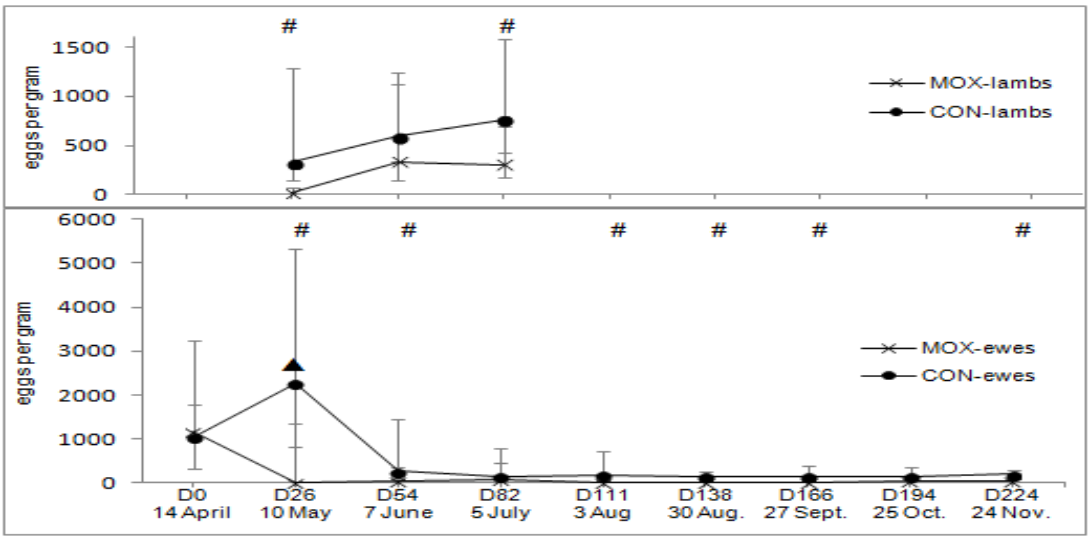


farm A a) lambs
b) ewes

Fig. 2:

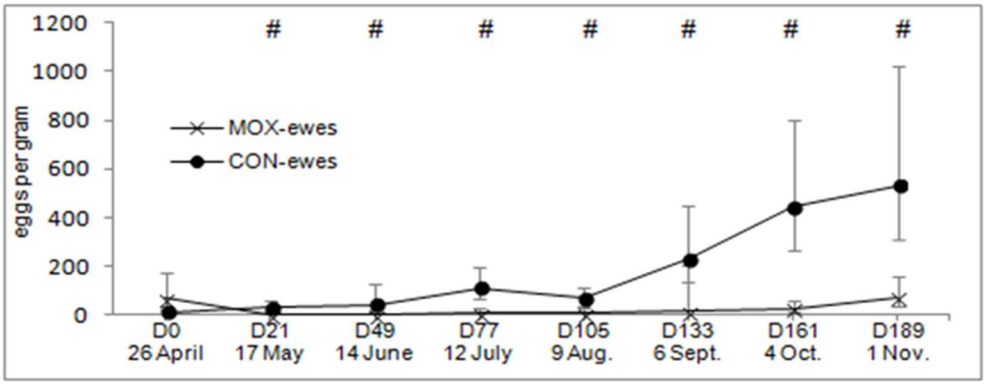
a)

b)



farm B a) lambs
b) ewes

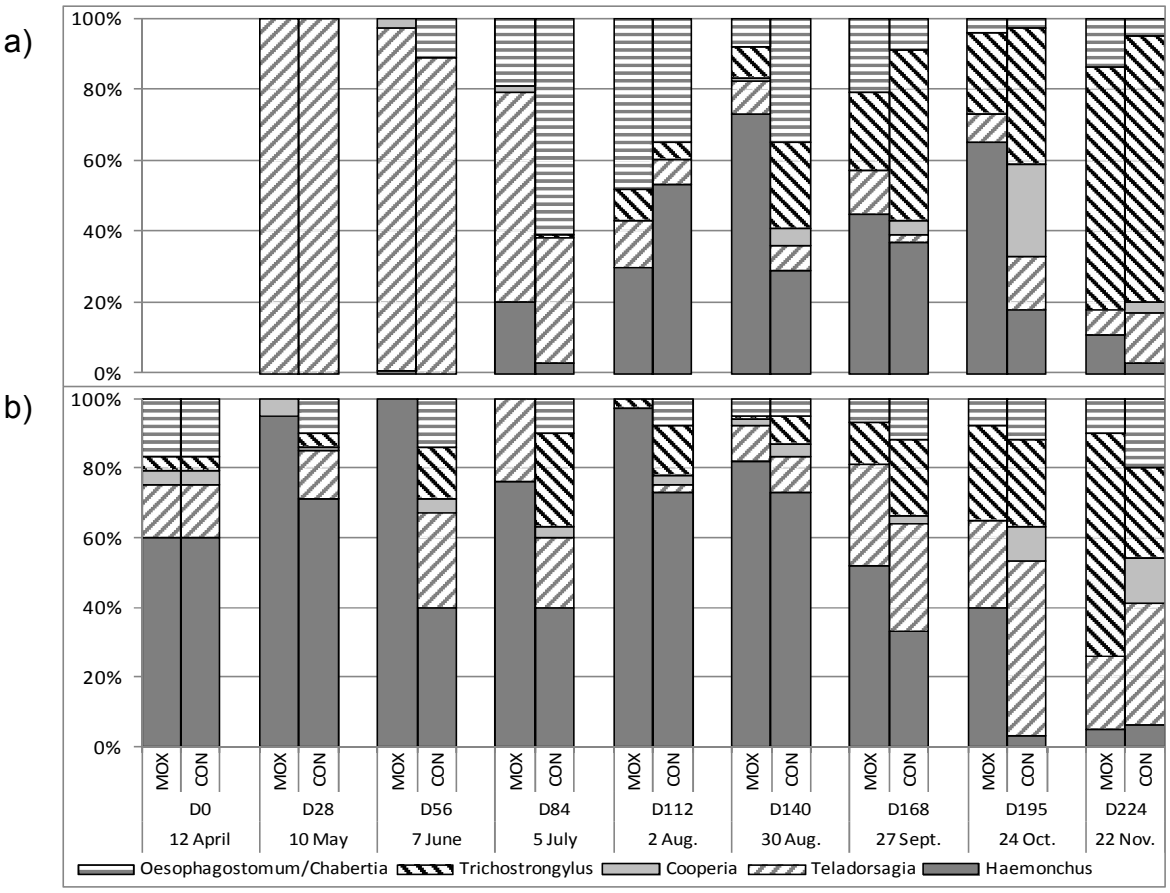
Fig. 3:



farm C

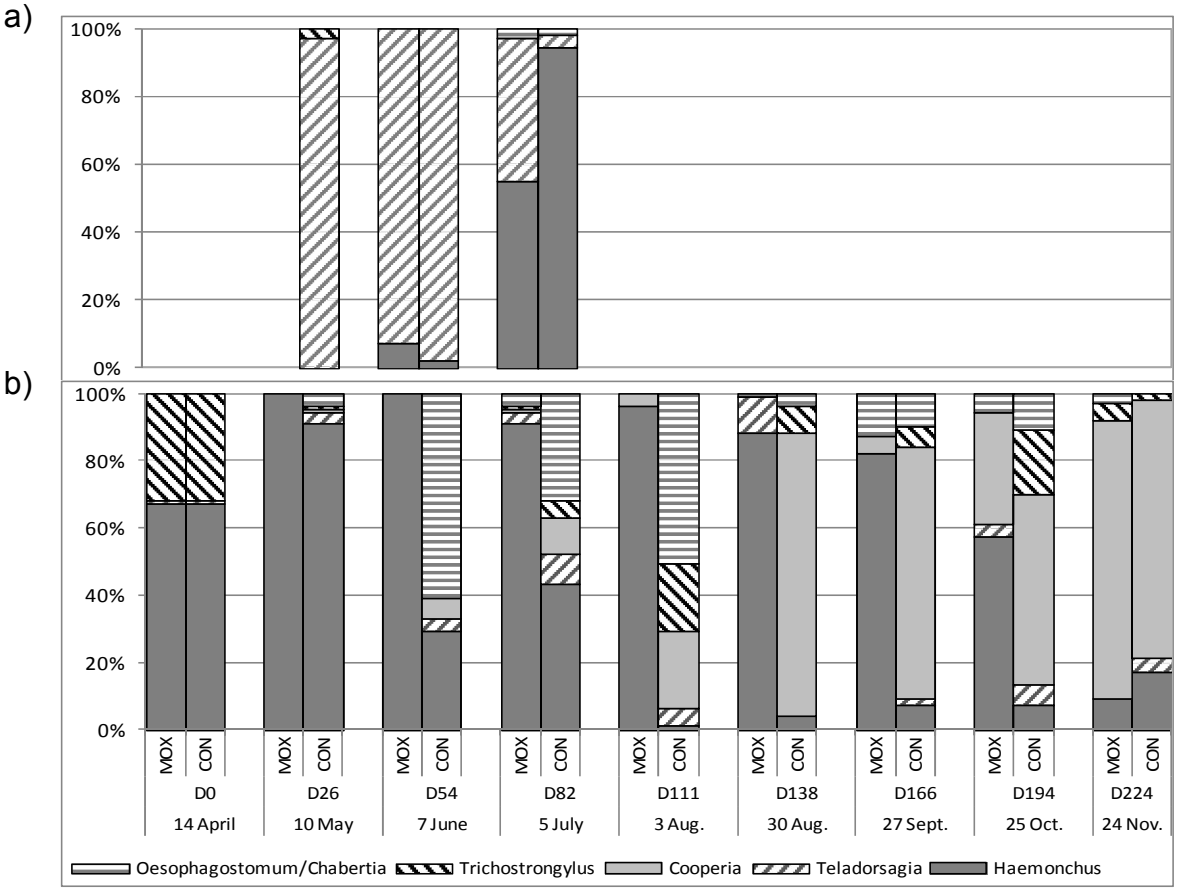
Figure 4-6: Differentiation of trichostrongyle third-stage-larvae in coprocultures on the group level

Fig. 4:



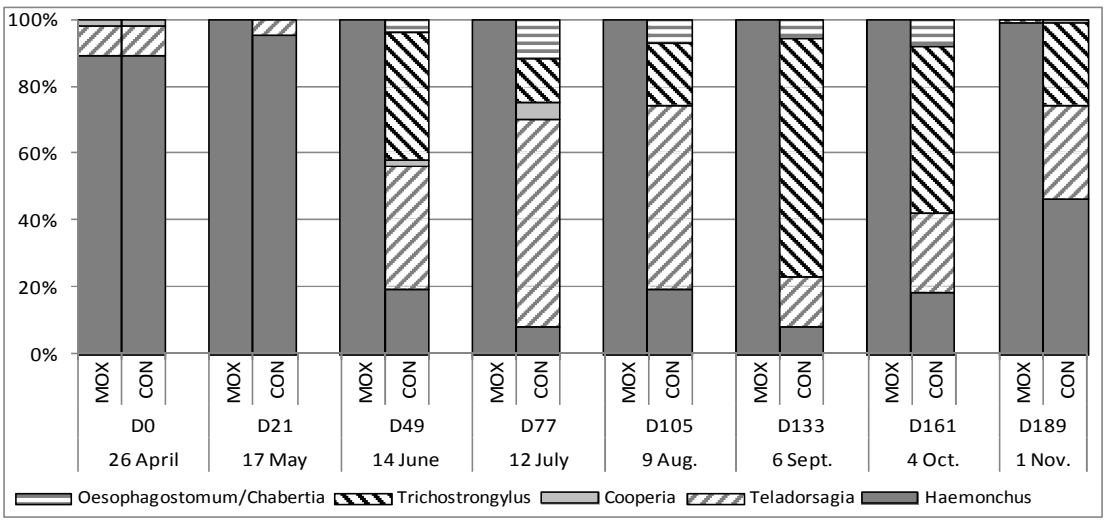
farm A a) lambs
b) ewes

Fig. 5:



farm B a) lambs
b) ewes

Fig. 6:



farm C

Fig. 7: Mean serum pepsinogen values of lambs on farm A

#: differences between MOX- and CON-lambs significant ($p<0.05$)

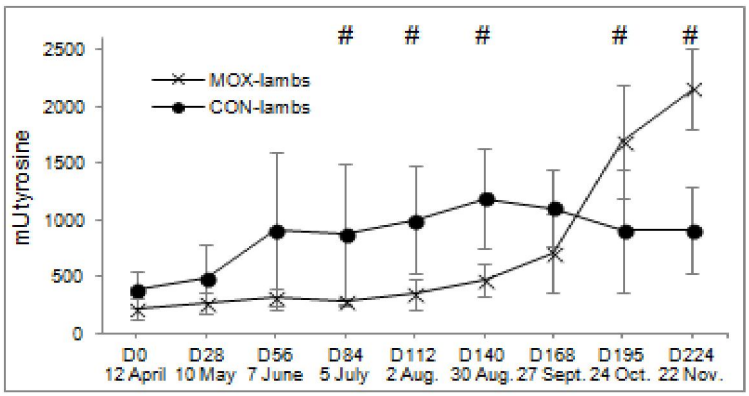
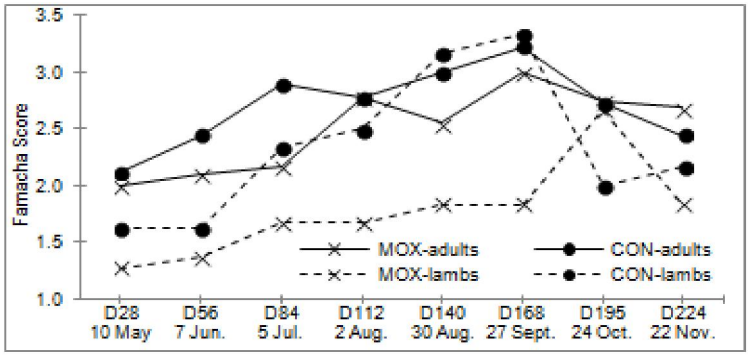


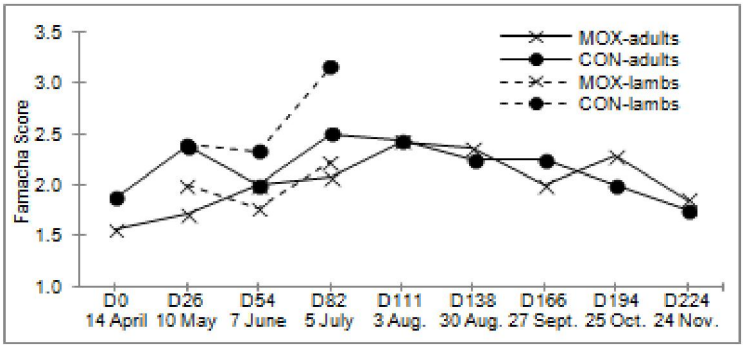
Figure 8-10: Mean FAMACHA scores of ewes and lambs

Fig. 8:



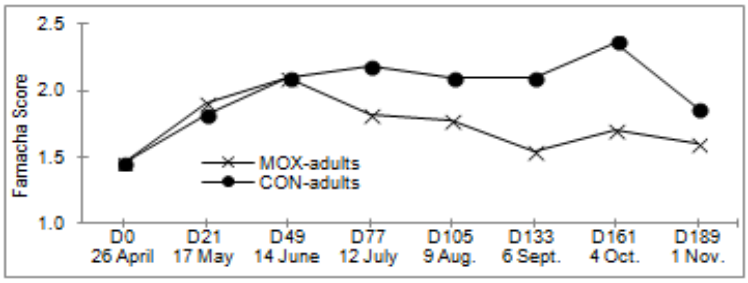
farm A

Fig.9:



farm B

Fig. 10:


Famacha Score
2.5
2.0
1.5
1.0
MOX-adults
CON-adults
D0
26 April
D21
17 May
D49
14 June
D77
12 July
D105
9 Aug.
D133
6 Sept.
D161
4 Oct.
D189
1 Nov.

farm C

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